Journal of Plant Biology, March 2000, 43(2) : 69-75

Growth Responses and Hyoscyamine Content of *Datura innoxia* under the Influence of Coal-Smoke Pollution

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This study evaluated the impact of pollution on growth responses in *Datura innoxia*. Coal-smoke emissions were produced by the Badarpur Thermal Power Plant in Delhi, India. At the polluted site, the size of roots and leaves as well as the number of branches and leaves per plant increased, but shoot lengths and leaf areas were lower, compared with control plants. The net photosynthesis rate, stomatal resistance, and the amount of pigments (chlorophyll a, b, and carotenoids) were less in pollution-affected plants, while stomatal conductance and intercellular CO_2 concentration were higher in these plants. Explants from both sites (polluted and non-polluted), grown in vitro on various combinations of auxin (2,4-D, NAA) and cytokinin (BAP, KN), showed the maximum response on a medium containing NAA (0.1 mg L^{-1}) with BAP (5.0 mg L^{-1}). Hyoscyamine content was higher in all parts (root, stem, leaf, and regenerants) of the polluted plants.

Keywords: coal-smoke pollution, *Datura innoxia*, explant culture, growth response, Hyoscyamine

Smoke pollution is a phytotoxic byproduct of combustion that causes invisible as well as visible damage in plants. It consists of gases, unburnt particles of coal, and fly ash that contains oxides of carbon, silica, alumina, and iron, plus certain metals such as arsenic, cadmium, berrylium, and selenium (Singh and Yunus, 2000). Soil, water, and air can become contaminated around the point source. The stress from regionally transported acidic depositions and the gaseous pollutants often cause structural and functional changes in plant communities, which may subtly degrade plant ecosystems. Air pollutants can affect various levels of plant organization, from an individual leaf to an entire ecosystem (Yunus and Iqbal, 1996). The present study investigated the impact of emissions from a thermal power plant on *Datura innoxia* Mill., Solanaceae. This plant is used as an anodyne, antiseptic, sedative, and cerebral depressant in the Indian systems of medicine. Its utility in treating catarrh, fever, diarrhoea, and skin diseases also is well-established.

MATERIALS AND METHODS

Mature *D. innoxia* plants (i.e., in the post-flowering stage) of comparable age and vigor were collected from areas around the Badarpur Thermal Power Plant (the polluted site), and from the Hamdard University Campus (the reference, or control, site). Delhi is in northern India, 160 km south of the Himalayas. It has a tropical semi-arid climate, with extremely hot summers and moderately cold winters. July to September is the typical monsoon rainy season. The Badarpur Thermal Power Plant is located on the Delhi-Haryana road, at 77° 22'E longitude and 28° 25 N latitude, and is 220 m above sea level. This plant comprises five units (three at 100 MW each and two at 210 MW each), and has a daily power-generating capacity of 720 MW from a consumption of about 10,000 tonnes of low-grade bituminous coal. It also emits 1450 tonnes of fly ash and 600 tonnes of SO_2 per day (details in Nighat et al., 1999). Hamdard University is located on the Badarpur-Mehrauli road, about 10 km west of the power station. Soils are saline and alkaline, and have similar compositions at both sites.

The roots, stems, and leaves of five mature plants from each site were measured in cm, and biomass was determined by drying the parts separately at 60°C for 48 h. Leaf area was calculated by using a leaf area meter (LICOR-3000, Lincoln, Nebraska, USA). Epidermal peels were obtained by treating small leaf pieces from the regions between midrib and leaf margins with 30% HNO_3, following the method of Ghouse and Yunus (1972). The epidermal structures were studied on a compound microscope.

An Infra Red Gas Analyzer (LI 6200 Portable Photosynthesis System, LICOR), was used for measuring

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photosynthesis rates, stomatal conductance and resistance, and intercellular CO_2 concentrations in the leaves. Mature individual leaves were clamped into the leaf chambers of the duly calibrated and checked apparatus for three minutes, then the parameters were analyzed by pressing the log button on the sensor head. Measurements were taken four times during the month, under sunshine between 0900 and 1100 h, on the five leaves from each plant and from three plants at each site.

The levels of chlorophylls *a* and *b*, and the carotenoids, in both control and polluted plants, were determined by placing 0.1-g leaf samples in 7 mL dimethyl sulfoxide (DMSO), then holding them for 4 h in an oven at 65°C. Afterward, 3 mL DMSO was added to 1.0-mL aliquots. Absorbance was recorded at 480, 510, 645, and 663 nm, using a spectrophotometer (Hiscox and Israelstam, 1979). The chlorophyll and carotenoid contents were estimated by the formulae of Duxbury and Yentsch (1956) and MacLachlan and Zalik (1963), respectively.

Nodal stem segments of plants from both sites were washed thoroughly with running tap water for 30 min, then with 0.3% cetrimide (ICI India) for 10 min. The washed segments were sterilized with 0.1% $HgCl_2$ for 6 min, followed by a final rinse with 70% alcohol for 1 min. These were then thoroughly washed with sterilized distilled water before implantation. We used Murashige and Skoog's (1962) medium, which was gelled with 0.62% agar (Qualigen, India) and pH-adjusted to 5.7 before being autoclaved for 15 min at 121°C and 15 kg cm^{-2}. An MS basal medium containing 1.5% sucrose was used for culture initiation. All the cultures were maintained at 25 ± 2°C in a culture room provided with a 14-h photoperiod (40 W cool-white fluorescent tubes and incandescent lamps, with a total intensity of 100 µmol $m^{-2}s^{-1}$).

Although regeneration could have been obtained on the MS basal medium, the cultures were transferred to various media combinations of auxin and cytokinin (2,4-D/Kn, NAA/Kn and NAA/BAP) and CH/glutamine, as indicated below:

1. MS medium (sucrose 4%) + 2,4-D (2.0 mg L^{-1}) + KN (1.0 mg L^{-1}) + CH (500 mgL^{-1})
2. MS medium (sucrose 3%) + NAA (0.2 mg L^{-1}) + KN (2.0 mg L^{-1})
3. MS medium (sucrose 3%) + NAA (0.1 mg L^{-1}) + BAP (5.0 mg L^{-1})
4. MS medium (sucrose 2%) + NAA (0.2 mg L^{-1}) + BAP (0.5 mg L^{-1}) + glutamine (50 mg L^{-1})

The morphogenic responses of the explants on each medium were recorded and compared after two, three, and four weeks.

Different concentrations of a hyoscyamine stock solution (ranging from 0.1 to 1.0 mL) were placed in separate tubes, and the individual volumes were made up to 0.1 mL with 95% ethanol. The solution in each tube was supplemented with 0.2 mL ammonia (10% v/v) and 2.5 mL chloroform, and warmed. The volume was then increased to 15.5 mL with more chloroform and, finally, to 40 mL with 6% acetic acid (in 5% ethanol). After vigorous shaking, the mixture was allowed to settle. A 0.5-mL portion was taken from the fractionated phase in each tube and evaporated to dryness. One-half mL of fuming HNO_3 was added to the residue and allowed to evaporate over a water bath. The residue was dissolved in 5 mL acetone (passed through anhydrous Na_2SO_4). To each tube, 0.05 mL of 3% methanolic potassium hydroxide was added. Five replicates of each concentration were scanned at 400-600 nm by altering the time interval from 2 to 8 min. The wavelength of the maximum absorbance (peak) was calculated, and sample absorbances were measured after 5 min at a wavelength of 556 nm, per Cromwell (1955).

The in-vivo leaves, stems, and roots, and the in vitro-regenerated plantlets were dried in a hot-air oven at 65°C for four days. The dried material was then powdered with a mortar and pestle. To the powdered sample, 0.5 mL of 95% ethanol and 0.05 mL of 10% (v/v) ammonia were added and heated gently. The sample was then percolated through a paper filter-lined funnel with more chloroform, at a flow rate of 1 drop/sec until the volume of the percolate reached 15.5 mL. To this, 24.5 mL of 6% acetic acid (in 5% ethanol) was added and shaken. A 0.5-mL portion of the upper layer was taken from this solution and evaporated to dryness. Standard curves were obtained as described above. The measured absorbances were interpolated with the standard curve (see also Srivastava et al., 1993).

RESULTS

The shoot axis was smaller but the root axis was markedly longer on a polluted *D. innoxia* plant, compared with an unpolluted control. Total plant heights were slightly reduced overall at the polluted sites, but the number of branches and leaves per plant, and leaf length and area were marginally greater than for control plants. However, biomass was generally larger

Table 1. Morphological parameters obtained from the polluted and non-polluted samples of *D. innoxia*. Values are means of 10 independent readings.

Parameters	Unpolluted	Polluted	% Variation
Root length (cm)	6.81 ± 0.45	13.13 ± 3.74	92.24*
Shoot length (cm)	30.61 ± 4.34	23.2 ± 3.16	24.20^{NS}
Branches per plant	2.80 ± 0.30	3.20 ± 0.84	14.28^{NS}
Leaf length (cm)	3.25 ± 1.02	8.34 ± 2.10	156.60*
Leaf area (cm^2)	18.98 ± 2.35	11.20 ± 2.18	40.99^{NS}
Leaves per plant	28.00 ± 4.46	26.80 ± 5.89	4.28^{NS}
Root biomass (g)	0.19 ± 0.07	1.39 ± 0.61	615.70*
Stem biomass (g)	0.83 ± 0.18	1.34 ± 0.63	61.44*
Leaf biomass (g)	1.21 ± 0.50	2.30 ± 1.23	90.08*

* = Significant at 1% level; NS = Non-significant.

in the polluted plants. Root biomass increased drastically, from 0.19 g to 1.39 g, while leaf biomass was nearly doubled, and showed significance at 1% level. Stem biomass also increased marginally (Table 1).

Stomata on both surfaces of the *Datura* leaf were of an anomocytic type. The epidermal cells had sinuous margins, the sinuations being more pronounced in the polluted leaves. The trichomes, present on both epidermis, had globular ends. Guard cells of stomata were shorter in the upper epidermis but larger in the

Table 2. Photosynthetic parameters obtained from the polluted and non-polluted samples of *D. innoxia*. Values are means of 60 independent readings.

Parameters	Unpolluted	Polluted	% Variation
Photosynthesis rate (μ mol CO_2 m^{-2}-s^{-1})	15.33 ± 2.41	6.48 ± 3.54	57.72**
Stomatal conductance (mmol H_2O m^{-2}-s^{-1})	0.55 ± 0.22	0.76 ± 0.33	38.18**
Intercellular CO_2 (ppm)	287.86 ± 14.52	308.46 ± 16.12	7.16*
Stomatal resistance (s cm^{-1})	0.78 ± 0.24	0.23 ± 0.05	70.51**
Chlorophyll *a* (mg/g fr. wt.)	0.77 ± 0.21	0.39 ± 0.12	49.35**
Chlorophyll *b* (mg/g fr. wt.)	0.47 ± 0.18	0.27 ± 0.06	42.55**
Carotenoid (mg/g fr. wt.)	0.56 ± 0.16	0.27 ± 0.08	51.78**

** = Significant at 1% level; * = Significant at 5% level.

Table 3. Morphogenetic responses of the polluted and non-polluted samples of *D. innoxia* noted after two, three, and four weeks on different culture media.

Medium	Cultures showing regeneration (%)		Leaves per regenerant		Maximum height of regenerant (cm)	
	Non-polluted	Polluted	Non-polluted	Polluted	Non-polluted	Polluted
	After two weeks					
A	66	85	13	7	2.75	2.25
B	98	66	26	8	3.50	2.20
C	95	66	20	9	3.50	3.00
	After three weeks					
A	98	98	22	14	3.75	3.80
B	95	75	43	30	3.50	3.75
C	98	66	38	17	4.25	4.50
	After four weeks					
A	99	98	30	22	4.00	4.00
B	98	85	52	40	3.75	4.00
C	98	75	47	25	4.50	4.75

A = MS medium (sucrose 3%) + NAA (0.1 mg L^{-1}) + BAP (5.0 mg L^{-1})
B = MS medium (sucrose 2%) + NAA (0.2 mg L^{-1}) + BAP (0.5 mg L^{-1}) + glutamine (50 mg L^{-1})
C = MS medium (sucrose 3%) + NAA (0.2 mg L^{-1}) + KN (2.0 mg L^{-1})

lower epidermis of polluted leaves, compared with those of the control. The mean length and width of stomatal pores were larger on both surfaces of the polluted leaves. Size and density of trichomes decreased on both surfaces, whereas stomatal density increased under polluted conditions.

The photosynthetic rate for control plants was 15.33 m moles $m^{-2}s^{-1}$. Although stomatal conductance had been significantly higher in the polluted plants, their photosynthetic rate was only about one-half that of the controls; this difference was significant at the 1% level. Stomatal resistance obviously had drastically declined in the polluted plants, which caused the intercellular CO_2 concentration to be increased (Table 2). Relative humidity and leaf temperatures were also higher in the polluted plants; their difference from the control being highly significant.

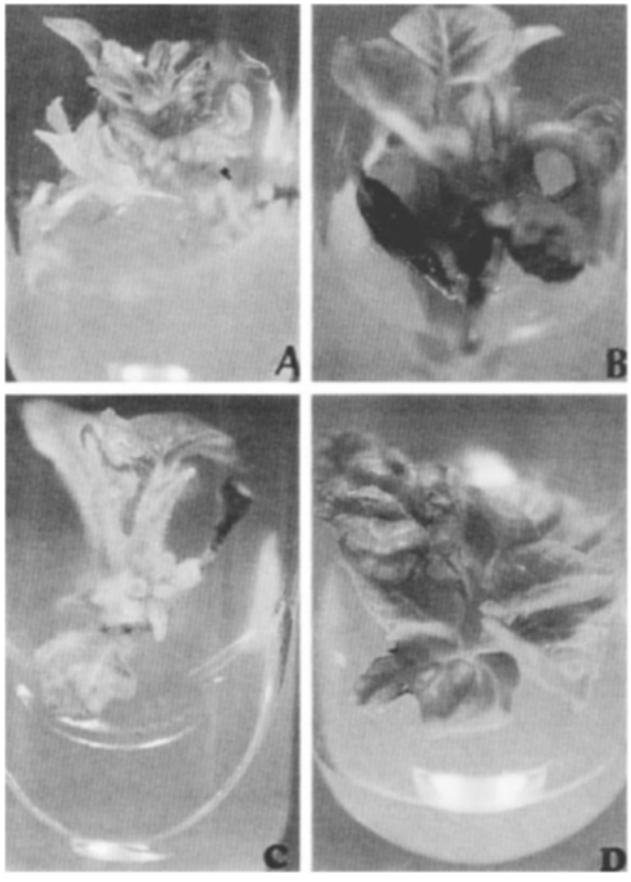

Figure 1. *D. innoxia*: (A-B), Regeneration of multiple shoots from mature stem segments on MS medium (sucrose 3%) + NAA (0.2 mg L^{-1}) + Kn (2.0 mg L^{-1}) after two weeks. More shoots and leaves developed from control explants (B), compared with the polluted explant (A). (C-D), Emergence of multiple shoots from stem segments after two weeks on MS medium (sucrose 3%) + NAA (0.1 mg L^{-1}) + BAP (5.0 mg L^{-1}). Half as many shoots developed from the polluted explant (C) than from the control (D).

Table 4. Comparative data on the estimated amounts of the alkaloid (hyoscyamine) in different parts of polluted and non-polluted samples of *D. innoxia*. The values are means of 10 independent readings.

Sample	Hyoscyamine (mg/500 mg leaf dry wt.)		
	Unpolluted	Polluted	%Variation
Root	0.06	0.90	1400.00*
Stem	1.04	1.74	67.30*
Leaf	0.85	1.78	109.40*
Regenerant	1.14	1.18	3.51^{NS}

* = Significant at 1% level; NS = Non-significant.

Nodal segments were obtained from plants at the control site and were implanted on MS medium to which 2,4-D, KN, and 4% sucrose had been added. These tissues showed swelling in about 10 to 12 days; callusing started by the third week. In polluted explants, callusing occurred after one month. When samples were placed on MS medium supplemented with 1) 0.1 NAA, 5.0 BAP, and 3% sucrose; or 2) 0.2 NAA, 2 KN, and 3% sucrose or 3) 0.2 NAA, 0.5 BAP, 50 mg L^{-1}glutamine, and 2% sucrose, regeneration for both control and polluted explants began within 10 to 12 days. However, regenerating efficiency of the polluted sample was generally low (Table 3).

The number of leaves derived from polluted samples was only about half as many, but their size was considerably larger than on the control explants (Fig. 1). Chlorophyll content in regenerated leaves was also lower in the polluted samples. The amount of hyoscyamine alkaloid in polluted *Datura* plants was higher in general, although the amount was almost negligible in the roots, compared with the stem and leaves. The estimated level of alkaloid content was at its maximum in fully grown regenerated plantlets (Table 4).

DISCUSSION

Under polluted conditions, *D. innoxia* plants exhibited shorter shoots and overall heights, although root lengths were significantly greater than in the control plants, increased. Plants at the polluted siteshowed no foliar injury and, in fact, had more branches and longer leaves. This increased plant growth might have been due to soil enriched with fly ash, which normally is extremely toxic, but at a low concentration may enhance the availability of essential elements, e.g., Cu, Zn, Fe, and Mn (Wong and Wong, 1990; Iqbal et

al., 2000).

The increase in root length demonstrates the ability of an individual species to establish itself under stress. Huang and Murray (1993) found that exposing *Triticum aestivum* to a low concentration of SO_2 stimulated root growth, thereby reducing the shoot-to-root ratio. Although SO_2, especially in high concentrations, is harmful to plants, it may initially improve growth rate at low concentrations. Sulphur accumulations in soil can enhance fertility of a sulphur-deficient soil; soil sulphur favors plant growth if soil nitrogen is not limiting (Ahmad et al., 1998, 1999). On the other hand, soil acidification facilitates heavy-metal uptake by plants, which may inhibit their growth (Iqbal and Khudsar, 2000). Under polluted conditions, the increases in plant biomass, particularly from roots, might have resulted partly from the increased accumulation of minerals and heavy metals in plant tissues growing in a fly ash-rich soil. Greater accumulation of metals in roots may indicate their low mobility into the rest of the plant (Petruzzelli et al., 1987).

Stomata were more frequent on leaves of polluted *D. innoxia*. This observation was consistent with those reported for *Catharanthus roseus* (Khan et al., 1990) and *Achyranthes aspera* (Dhir et al., 1999). The lower frequency and shorter lengths of trichomes may have been a result of heavy deposition of particulates on young leaves, which could suppress trichome development and growth (Rangarajan et al., 1995; Dhir et al., 1999). SO_2 and heavy metals can reduce carbohydrate production in plants, measured as net assimilation rate or net photosynthesis (Mehindirata et al., 1999; Nighat et al., 1999).

SO_2 may also directly affect photosynthesis because its various intra-cellular derivatives and photo-induced, oxidizing free radicals interfere with the carbon metabolic pathway (Malhotra and Khan, 1984). The decrease in net photosynthesis caused by SO_2 is primarily due to the breakdown of chloroplast activity rather than to any change in mitochondrial or peroxisomal activity, or stomatal closure (Furukawa et al., 1980). Higher concentrations of SO_2 inhibit the Calvin cycle enzymes (Tanaka et al., 1984). Deposition of dust also prevents stomata from closing; this condition tends to increase water loss and enhance uptake of gaseous air pollutants (Fluckiger et al., 1979). These effects eventually hamper photosynthesis (Mishra and Shukla, 1986). Unlike in many other plant species, stomatal conductance tended to be higher in *D. innoxia* plants at the polluted site. This could have been caused by water stress, which then prevented stomatal closure. However, these polluted plants showed declining photosynthetic rates, while intercellular CO_2 levels obviously increased.

Coal-smoke pollution markedly reduced the amounts of chlorophylls *a* and *b*, and carotenoids in *D. innoxia*. These responses were also reported for numerous other species (Wali et al., 1997; Dhir et al., 1999; Husen et al., 1999; Nighat et al., 1999). The reduction in chlorophyll may be attributed to SO_2-induced removal of Mg^{2+} ions by two atoms of H from chlorophyll molecules. As a result, chlorophyll is converted to phaeophytin, thereby changing the light spectrum characteristics of the molecule (Rao and Le Blanc, 1966; Shimazaki et al., 1980; Suwannapinunt and Kozlowski, 1980). The reduction in chlorophyll pigment in *D. innoxia* was not associated with any symptoms of foliar injury. This indicates that synthesis of chlorophyll pigments had decreased (Shimazaki et al., 1980). In addition, the presence of heavy metals may reduce synthesis or accelerate degradation of chlorophyll (Singh et al., 1994).

In the present study, regeneration efficiency of *D. innoxia* explants markedly declined for plants growing under pollution stress; this agrees with the responses seen in *A. aspera* (Dhir et al., 1999) and *Sida acuta* (Wali et al., 1997). Although the regenerants of polluted explants had fewer leaves per plant, plant heights hardly differed from those of the controls. Much research has focused on developing metal-tolerant plants (Meredith, 1978; Mishra and Gedanu, 1989; Chakravarty and Srivastava, 1992, 1997), but the regeneration potential of plants, as it relates to air pollution, is yet to be fully understood.

The alkaloid (hyoscyamine) content was considerably higher for polluted plants (root, stem, and leaf) in vivo, as well as for the regenerated plantlets. This increase in alkaloid content may indicate a chemical adaptation or a defensive/protective strategy under stress. The amount of hyoscyamine was quite low in roots, but relatively higher in stems and leaves. One explanation may be that hyoscyamine, which is synthesized in the roots, is transformed to 6-OH hyoscyamine and then to scopolamine (hyosine) during its migration to the stalk (Maldonado-Mendoza et al., 1992). Regenerated plantlets showed a maximum level of hyoscyamine possibly because the controlled environmental and nutritional conditions ensured a continuous yield of metabolites.

Besides the physiological status of the explant and cultures, physical factors such as light and temperature have a significant bearing on plant development (Srivastava et al., 1993). The present study suggests that, despite several adverse effects of the pollution

on plant growth, production of secondary metabolites such as hyoscyamine alkaloid was enhanced in both in vivo and in vitro samples. This response might represent a state of chemical adaptation, or a defensive mechanism toward environmental stress. This is significant, from a commercial point of view. Because these plants were able to withstand pollution stress sufficiently, their introduction on degraded lands may also help in reclaiming problem soils.

ACKNOWLEDGEMENTS

The authors thank their colleagues for laboratory assistance and Mr. Zakir Ahmad of the Botany Department, Jamia Hamdard, New Delhi, for help with computer typing.

Received December 27, 2000; accepted March 13, 2000.

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